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Egloff, S ; Schneeberger, M ; Gobeli Brawand, Stefanie ; Krudewig, Christiane ; Schmitt, S ; Reichler, Iris ; Peterhans, Sophie

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# *Brucella canis* infection in a young dog with epididymitis and orchitis

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## Abstract

The following case report describes the clinical and diagnostic procedure for suspected brucellosis infection in a dog. A 21 month old intact male Border Collie was presented with an enlarged right testicle and epididymis. The dog was imported to Switzerland from Germany at the age of three months, but was never abroad since then. Clinical and laboratory diagnostic investigation included bacteriology and histology. An initial serological evaluation by means of rapid slide agglutination test (RSAT) was negative. Repeated examination of the same serum by a chromatographic immunoassay (ICT) revealed a positive result. *Brucella canis* infection was confirmed by culture. The present case is intended to underline the importance of the suspected diagnosis of 'brucellosis' in the presence of reproductive tract problems in dogs. In addition, *Brucella canis* has zoonotic potential and it is imperative to comply with strict hygiene management.

**Key words:** *Brucella canis*, brucellosis, zoonosis, castration, chromatographic immunoassay, rapid slide agglutination test

## *Brucella canis* Infektion eines jungen Hundes mit Epididymitis und Orchitis

Der nachfolgende Fallbericht beschreibt das klinische und diagnostische Vorgehen bei Verdacht auf Brucellose bei einem Hund. Ein 21 Monate alter intakter Border Collie Rüde wurde mit vergrössertem rechten Hoden und Nebenhoden vorgestellt. Der Hund wurde mit drei Monaten aus Deutschland in die Schweiz importiert, war aber ansonsten nie im Ausland. Es erfolgte eine klinische sowie labordiagnostische Untersuchung inklusive Bakteriologie und Histologie. Eine erste serologische Abklärung mittels rapid slide agglutination test (RSAT) war negativ. Eine wiederholte Untersuchung desselben Serums mittels eines chromatographischen Immunoassay (ICT) ergab ein positives Resultat. Mittels kulturellem Nachweis wurde der Verdacht einer Infektion mit *Brucella canis* bestätigt. Der vorliegende Fall soll die Wichtigkeit der Verdachtsdiagnose 'Brucellose' bei Vorliegen von Problemen des Reproduktionstraktes bei Hunden unterstreichen. Desweiteren hat *Brucella canis* zoonotisches Potential und es ist zwingend ein strenges Hygienemanagement einzuhalten.

**Schlüsselwörter:** *Brucella canis*, Brucellose, Zoonose, Kastration, chromatographischer Immunoassay, rapid slide agglutination test

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## Introduction

Canine brucellosis is caused by *Brucella (B.) canis*, a Gram-negative and facultative intracellular bacterium. It is considered a zoonotic pathogen, however, the importance as human pathogen remains unclear. Classical symptoms are late abortions and stillbirths in bitches, epididymitis, orchitis and sperm abnormalities in males and infertility in both sexes<sup>23</sup>. Furthermore, unspecific signs such as lymphadenitis, uveitis and discospondylitis have been reported<sup>12,20</sup>. Infected dogs often show

no clinical signs at all, but have intermittent bacteremia that may last for years and thus act as a continuous source of infection<sup>13,17</sup>. Transmission is mainly through vaginal secretions and semen, but also through urine<sup>23</sup>. It has been suggested, that stray dogs in the Mediterranean area and eastern Europe serve as a reservoir<sup>2,5,22</sup>, therefore the increasing import of dogs from endemic countries seems to pose a risk for re-introducing of canine brucellosis in central Europe<sup>4</sup>. In the last ten years, different cases of both female and male dogs in northern and southern Europe have been reported, including a

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kennel outbreak in Sweden in 2013<sup>16</sup> and in Austria in 2012<sup>12</sup>, as well as single cases in Germany, Italy, UK and Sweden<sup>7,8,14</sup>. The laboratory diagnosis of *B. canis* infection is difficult and depends upon the stage of the disease. Two weeks post-infection, antibodies can be found and thus be detected via serology<sup>23</sup>. However, the sensitivity and specificity depend strongly on the chosen serological test and therefore, the final diagnosis should be confirmed by *B. canis* isolation by culture or PCR diagnostics<sup>18,19</sup>.

Here, we report a rare case of *B. canis* infection in a young dog with unilateral epididymitis and orchitis.

## Case report

On March 2nd 2017, a 21 months old male intact Border Collie was referred from the private veterinarian to the animal hospital of the Vetsuisse Faculty of Zurich due to testicular and epididymal enlargement (Figure 1). The owner mentioned that the dog showed pain on palpation of the lower abdomen and only wanted to lie on cold ground, but was in an overall good general condition. He was imported from Germany at the age of three months and has never been in another country. In the breeding kennel there has been no history of *B. canis* infection. The parent animals are reported as being tested negative for *B. canis*. He was kept as a single family dog, was never bred or had been at any dog shows and had been vaccinated and dewormed regularly.



**Fig. 1:** Examination of the testicles in a 21 months old Border Collie revealed an enlarged right testicle and epididymis with visible reddening of the scrotum. On palpation the right testis was enlarged, hardened and painful, while the palpation of the left side did not reveal abnormal findings.

The dog was presented in a slightly reduced general condition, tachycardic (HF 124/min), nervous and panting, but with a normal rectal temperature (38.6 °C). The right testicle and epididymis were enlarged, hardened and painful, while the palpation of the left side did not reveal abnormal findings. Both testicles were still movable within the scrotum. The palpation of the prostate was without any abnormal changes.

A complete serum biochemistry and complete blood count were done at the in-house laboratory of the Vetsuisse Faculty as well as a urine analysis from cystocentesis urine. Remarkable changes were a leukocytosis ( $31.1 \times 10^3/\mu\text{l}$  [ref.  $4.7\text{--}11.3 \times 10^3$ ]) with neutrophilia ( $25.3 \times 10^3/\mu\text{l}$  [ref.  $2.50\text{--}7.44 \times 10^3$ ]), as well as a slightly increased blood albumin level (42g/l [ref. 29–37g/l])<sup>6</sup>. There were no remarkable findings in the urine analysis. A blood sample was sent for serological detection of antibodies against *B. canis*.

Ultrasonographically, anechoic free fluid surrounding the right epididymis was detected, as well as enlargement, irregular margination and severe heterogeneity in the echotexture of the right epididymis (Figure 2 & 3). The right testicular parenchyma was enlarged and showed decreased reflectivity of the rete testis. Prostate and urinary bladder were within normal limits.

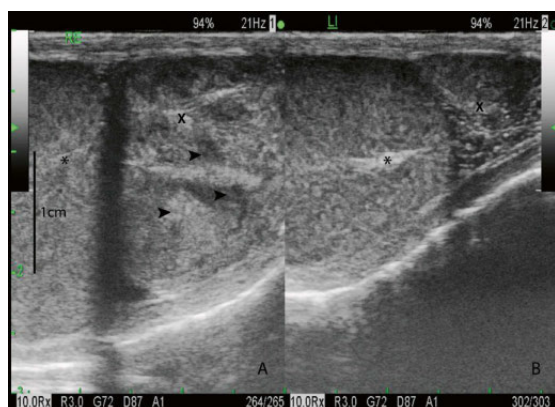
## Surgical castration

Surgical castration (scrotal ablation, closed castration) with histological as well as bacteriological investigation of the affected epididymis and testicle was recommended and executed on the same day.

Castration was performed as scrotal ablation, creating an elliptical incision at the transition from pigmented to non-pigmented scrotal skin. On the affected side the transition from macroscopically unaffected to inflamed skin was removed as marching. In this case it was decided to perform closed castration, without incision of the vaginal tunic. Testes were advanced and ligated twice, distally with an anchoring ligature around the Musculus cremaster, proximally with a mass ligature. Before cutting and removing of the testicle a clamp was set to prevent the stump from leaking. After examining the stump for bleedings, the inguinal canal was closed with Sultan sutures. Subcutis and cutis were closed with subsequent single knot sutures.

Intraoperative findings were a remarkable enlarged epididymis of the right side with purulent effusion at the cutting side as well as a visibly increased blood circulation of the affected scrotal skin. On the left side, no macroscopic changes were observed.





**Fig. 2:** Longitudinal ultrasonographic image (A) of the right compared to (B) the left testis (\*) and epididymis (x) at the same projection size in a Border Collie. The right epididymis is markedly enlarged, irregularly delineated and heterogeneous changed (arrow head) compared to the one on the left side. The parenchyma of the right testis is enlarged but without any remarkable changes in shape or structure.



**Fig. 3:** Longitudinal ultrasonographic image of the right epididymis in a Border Collie. Anechoic free fluid surrounding the epididymis (arrow), its enlargement and the heterogeneous echotexture (arrow head) are well visible.

Biopsies from the resected right testicle and epididymis as well as samples of the purulent secretion were taken for bacteriological culture and for histology. Samples for histology were put in 10% buffered formalin, fixed for 48 hours and embedded in paraffin blocks.

Postsurgically, analgetic treatment with Carprofen at 4mg/kg SID p.o. and antibiotic therapy with Enrofloxacin at 10mg/kg SID p.o. was started. Due to rapid recovery, the dog went home the same day.

## Further laboratory examinations

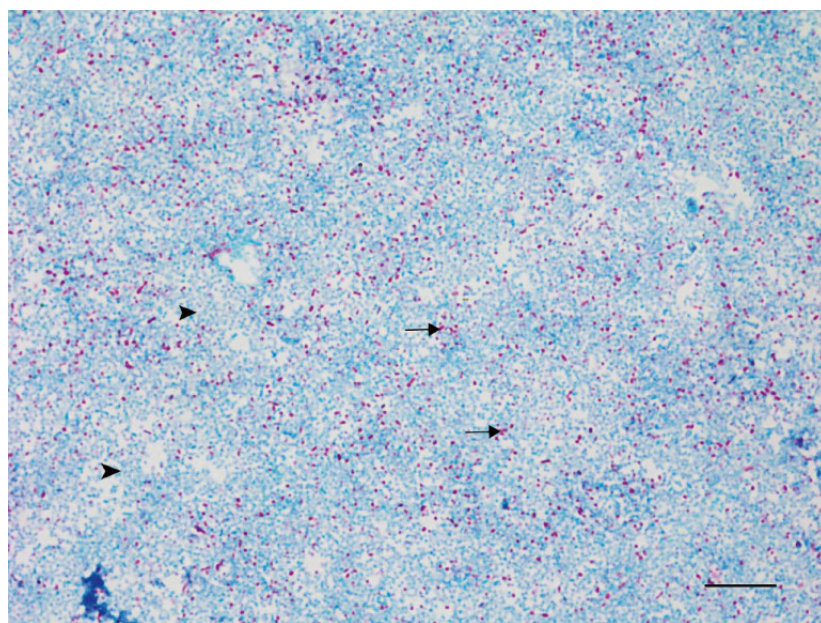
### Microbiological examinations

An epididymal tissue sample, a swab with purulent material and a urine sample were submitted for bacteriological examination. The tissue sample and the swab were pooled, the urine was examined separately. The

specimens were cultivated on solid Columbia sheep blood agar (Thermo Fisher Scientific, Basingstoke, UK) and incubated for 96 hours at 37°C under an aerobic atmosphere. The agar plates were monitored for bacterial growth after 48, 72 and 96 hours. After 96 hours of incubation grey to translucent, convex and round colonies were detected from the pooled tissue and swab sample. Catalase, oxidase and urease testing of the grown colonies were positive, latter reacted under two hours. A modified Ziehl-Neelsen staining<sup>3</sup> (Stamp's modification) showed slightly stained small, red coccobacilli (Figure 4). The presumptive *Brucella* spp. colonies were confirmed by PCR as *B. canis*<sup>11</sup>. No colonies were grown from the urine specimen.

### Serology

In order to detect antibodies against *B. canis* a serum sample was taken. The specimen was tested negative using a rapid slide agglutination test (RSAT) (Canine Brucellosis Antibody Test Kit D-Tec, Zoetis, USA). Subsequently, the same serum sample and another one taken three weeks after the first one were submitted to the Institute of Veterinary Bacteriology in Bern (ZOBA). The samples were analyzed using a chromatographic immunoassay (ICT) (Rapid Canine Brucella Ab Test Kit, Bionote, Republic of Korea) and were both tested positive. Four subsequent serum samples taken during the following five months were tested either using the ICT or the RSAT as the chromatographic immunoassay was temporarily not available. All samples were tested negative.



**Fig. 4:** Stamp's modified Ziehl-Neelsen staining of *B. canis*, isolated from the epididymal tissue sample of a Border Collie. Shown are slightly stained (arrow, pink cells) as well as unstained cells (arrowhead, blue cells) of the *B. canis* isolate, bar = 10 µm.

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### Histological examination

Routinely, H&E staining of the FFPE (formalin fixed, paraffin embedded) material was performed. Main findings of the right testicle and epididymis were a severe multifocal to confluent lymphoplasmacytic to necro-suppurative epididymitis and mild multifocal necro-suppurative orchitis (Figure 5 & 6). Gram and Ziehl-Neelsen staining did not reveal infectious agents. In comparison no abnormal findings on the left testicle and epididymis was found.

### Follow up treatment

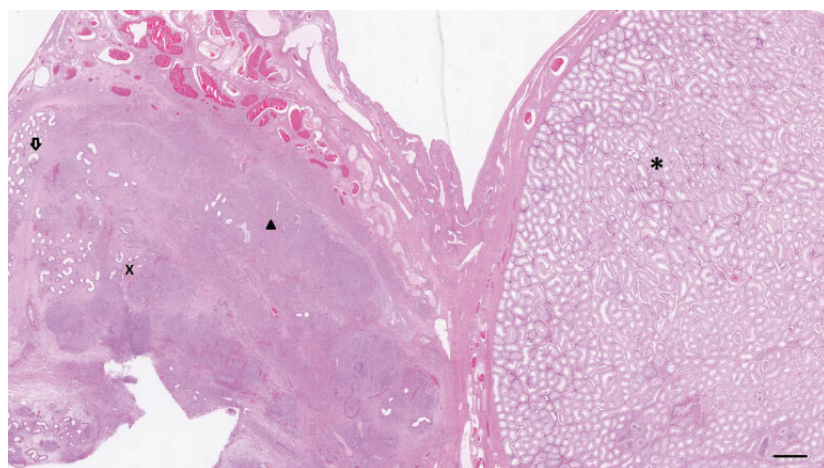
Seven days after surgery, when the diagnosis *B. canis* had been confirmed, treatment with Doxycycline at 10mg/kg BID p.o. for four weeks combined with Enrofloxacin at 10mg/kg SID p.o. for two more weeks and Strepto-

mycin 20mg/kg SID s.c. for seven days was initiated<sup>10</sup>. Because of the risk of developing urinary casts due to renal tubular necrosis as a side effect of the aminoglycosides, urine analysis as well as monitoring urea and creatinine blood concentrations were recommended at one and two weeks after initiation of treatment. The urine analysis did not show any remarkable changes or signs of casts. Additionally, bacteriological hemoculture or PCR in three-month-intervals until twice tested negative was advised, and antibody titer identification in six to nine month intervals was recommended. In the current case the dog has been tested serologically negative one, two, four and six months after being diagnosed. Because another possible location of manifestation of *B. canis* is the eye, uveitis and retinitis have been ruled out by an ophthalmologist.

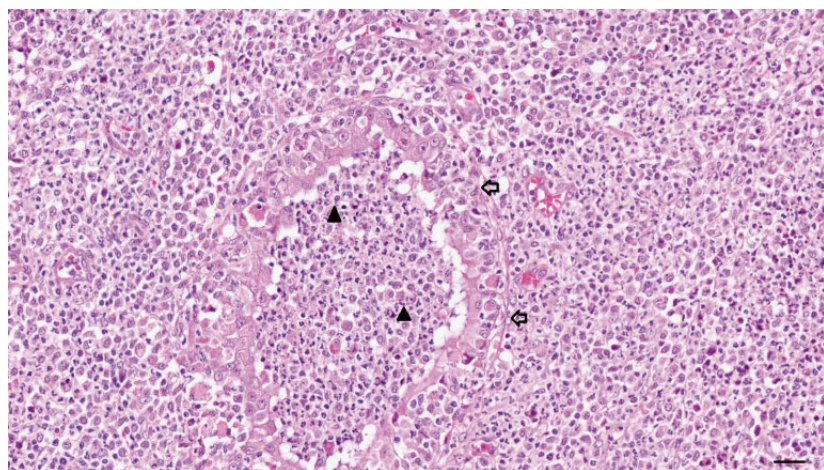
### Discussion

In recent years several cases of *B. canis* positive dogs have been reported all over Europe. Persistence of canine brucellosis in stray dog populations in the Mediterranean area as well as in Eastern Europe is very likely<sup>7,9</sup>. Most outbreaks in Western and Central Europe are due to the import of dogs from endemic regions, as happened in 2013 in an outbreak in a Swedish kennel after the import of an infected dog from Spain<sup>16</sup> or in 2016 in the UK after the import of a dog from Romania<sup>15</sup>. However, often the source of infection remains unclear, such as in an outbreak in a kennel in Austria with no epidemiological history of contact with imported dogs or stray dogs abroad<sup>12</sup>. Also, in the current case neither import from endemic regions, nor travelling to these regions is reported and therefore the transmission pathway remains unknown.

The final diagnosis of canine brucellosis should be based on direct laboratory diagnosis, such as cultural detection or PCR diagnostics<sup>18,23</sup>. In the current case, samples of the reproductive tract were tested. In literature, whole blood is considered the specimen of choice for *B. canis* isolation, because bacteremia is prolonged and lasts for about six months. Furthermore, genital tract samples are often secondary contaminated due to growth of commensal bacteria, whereas blood samples can be taken aseptically<sup>18,19</sup>. Bacteremia starts between two and four weeks post-infection. Shedding of *B. canis* through urine appears between four to eight weeks post-infection and has been reported as being positive in animals with negative blood culture results<sup>23</sup>. However, urine culture was negative in the current case. A long incubation time is crucial for *B. canis* to grow, as in our case, where colonies appeared after only four days. Nevertheless, because of the time-consuming culture and the often contaminated specimens, the isolation of *B. canis* is not



**Fig. 5:** Histopathologic picture illustrating the testis (\*) and the epididymis (X). Typically for *Brucella* spp. infection the epididymis is more affected than the testis. Arrowhead: Neutrophils obscuring 80% of the epididymal architecture, arrow: remnant epididymal ductules, H&E-Staining, bar = 500 µm.



**Fig. 6:** Epididymis, close up. Ductule and surrounding tissue are filled with neutrophils characteristic for an acute necro-suppurative Epididymitis. Arrowhead: Neutrophils within the ductular lumen, arrow: ductular epithelial cells, H&E-Staining, bar = 20 µm.

always possible and for screening purpose serology is an important tool<sup>18</sup>. Currently, three different serological tests are mainly used for *B. canis* antibody detection: the RSAT, the agar gel immunodiffusion test (AGID) and the ICT. All of those methods detect antibodies against cell wall antigens of rough *Brucella* spp.<sup>18</sup>. The serum of the current case was first tested negative by RSAT. A re-examination of the same serum sample by ICT, however, revealed a positive result. Comparison of both tests by Keid et al. 2015 showed a higher diagnostic sensitivity for the ICT with 89.6% compared to the RSAT with 75%<sup>18</sup>. A possible explanation are the different detection methods for the immunoglobulins in these two tests; in ICT each antibody that binds to antigen can be seen by using a secondary antibody, whereas the visualization of the antibody-antigen reaction in the agglutination assay requires multiple binding reactions<sup>18</sup>. A reassessment of a negative tested serum in presumptive *Brucella* positive dogs may be worthwhile, not only because of differences in sensitivity between various tests, but also due to the fact, that no serological test is completely accurate until twelve weeks post-infection<sup>23,24</sup>.

Susceptibility testing of *Brucella* spp. can be done using the minimum inhibitory concentration (MIC) system according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M45-A2)<sup>1</sup>. It is, however, not performed routinely in veterinary diagnostics<sup>21</sup>. A combination therapy with tetracycline and streptomycin is described to be the most successful treatment regimen for *B. canis* infections<sup>10</sup>. Enrofloxacin was additionally chosen before knowing the cause and continued until the optimal medication has been confirmed.

If a case of *Brucella canis* infection is suspected, the affected dog should be quarantined immediately. The patient should be treated only with scrubs, gloves and masks because of the zoonotic potential of the disease. Serological tests and microbiological cultures of blood or affected tissue should be carried out. If tested positive, the dog should be neutered and treated as described above to minimize shedding. Additionally it should be recommended that parent animals or dogs bred with infected dogs are tested as well. Quarantine is advised until twice tested negative by PCR or culture. Contact dogs should be tested as well, at least serologically<sup>10</sup>. In the present case, parent and further contact dogs have been tested negative for *B. canis*, therefore the source of infection remains unknown.

Common disinfectants such as quaternary ammonia and iodides can be used for cleaning<sup>10,23</sup>. Infection control in kennels is described in detail by Wanke et al. 2004<sup>23</sup>.

Insidiously, many dogs with brucellosis remain asymptomatic and therefore untreated; as a result, intermittent bacteremia can persist for years and the pathogen can be spread unnoticed<sup>13,23</sup>. Even though, *B. canis* infections are scarce in Western and Central Europe it should always be considered as a differential diagnosis for reproductive or chronic inflammatory diseases.

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## Infection à *Brucella canis* chez un jeune chien souffrant d'épididymite et d'orchite

Le rapport de cas suivant décrit la procédure clinique et diagnostique en cas de suspicion d'infection par la brucellose chez un chien. Un Border Collie mâle intact de 21 mois a été présenté avec un grossissement du testicule et de l'épididyme droits. Le chien avait été importé d'Allemagne en Suisse à l'âge de trois mois, mais n'avait si non jamais été à l'étranger depuis lors. Des examens diagnostiques cliniques et de laboratoire, notamment bactériologie et histologie ont été effectués. Une première évaluation sérologique au moyen du test d'agglutination rapide sur lame (RSAT) était négative. Un examen ultérieur du même sérum par une immunanalyse chromatographique (ICT) a révélé un résultat positif. L'infection à *Brucella canis* a été confirmée par culture. Le présent cas souligne l'importance du dia-

## Infezione da *Brucella canis* in un giovane cane con epididimite e orchite

Il seguente studio descrive la procedura clinica e la diagnosi per una sospetta infezione da brucellosi in un cane. Un Border Collie maschio di 21 mesi intatto è stato presentato con un ingrossato testicolo destro ed epididimo. Il cane è stato importato in Svizzera dalla Germania all'età di tre mesi, e mai trasportato all'estero da quel momento. Sono state effettuate indagini cliniche e di laboratorio, comprese batteriologia e istologia. Un'iniziale valutazione sierologica mediante il rapid slide agglutination test (RSAT) è risultata negativa. L'esame ripetuto dello stesso siero mediante immunodosaggio cromatografico (ICT) ha rivelato un risultato positivo. L'infezione da *Brucella canis* è stata confermata dalla coltura. Il presente caso intende sottolineare l'importanza della sospetta diagnosi di "brucellosi" in

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gnostic présumé de «brucellose» en présence de problèmes de l'appareil reproducteur chez le chien. De plus, *Brucella canis* a un potentiel zoonotique et il est impératif d'appliquer des mesures d'hygiène strictes.

**Mots-clés:** *Brucella canis*, brucellose, zoonose, castration, dosage immunologique chromatographique, rapid slide agglutination test

presenza di problemi del tratto riproduttivo nei cani. Inoltre, *Brucella canis* ha un potenziale zoonotico ed è imperativo rispettare una rigorosa gestione dell'igiene.

**Parole chiave:** *Brucella canis*, brucellosi, zoonosi, castrazione, immunodosaggio cromatografico, rapid slide agglutination test

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